Immunology 2004 **112** 38–43 doi: 10.1111/j.1365-2567.2004.01869.x

FAST TRACK

Normal human pregnancy is associated with an elevation in the immune suppressive $CD25^+$ $CD4^+$ regulatory T-cell subset

DAVID A. SOMERSET,* YONG ZHENG,† MARK D. KILBY,* DAVID M. SANSOM† & MARK T. DRAYSON‡

**Department of Fetal Medicine, Division of Reproduction and Child Health, University of Birmingham, Birmingham Women's Hospital, †MRC Center for Immune Regulation, University of Birmingham, ‡Department of Immunology, Division of Immunity and Infection, University of Birmingham, United Kingdom*

SUMMARY

$CD4^+$ $CD25^+$ T regulatory cells (T_{Reg}), suppress antigen-specific immune responses and are important for allograft tolerance. During pregnancy the mother tolerates an allograft expressing paternal antigens (the fetus) requiring substantial changes in immune regulation over a programmed period of time. We analysed whether immune-suppressive T_{Reg} cells were altered during pregnancy and therefore might play a part in this tolerant state. The presence of T_{Reg} cells was assessed in the blood of 25 non-pregnant, 63 pregnant and seven postnatal healthy women by flow cytometry. We observed an increase in circulating T_{Reg} cells during early pregnancy, peaking during the second trimester and then a decline postpartum. Isolated $CD25^+$ $CD4^+$ cells expressed *FoxP3* messenger RNA, a marker of T_{Reg} cells, and suppressed proliferative responses of autologous $CD4^+$ $CD25^-$ T cells to allogeneic dendritic cells. These data support the concept that normal pregnancy is associated with an elevation in the number of T_{Reg} cells which may be important in maintaining materno-fetal tolerance.

Keywords anergy: suppression, tolerance; regulatory T cells (Treg); T cells

INTRODUCTION

$CD25^+$ $CD4^+$ regulatory T cells (T_{Reg}) are a specialized subset of T cells involved in preventing autoimmunity.[1–3] Studies have also demonstrated that T_{Reg} can prevent graft rejection,[4,5] indicating that these cells are potent suppressors of T-cell responses. T_{Reg} arise in the thymus as a result of altered negative selection and are present from fetal life onwards. However, there are suggestions that T_{Reg} may also develop in response to antigenic stimulation under non-immunogenic circumstances.[6]

The mechanism by which T_{Reg} suppress responses is uncertain. Both cell contact and cytokine-based mechanisms have been proposed and roles for interleukin-10 and transforming growth factor-β (TGF-β) have been suggested.[7–12] Interestingly, recent data indicate that TGF-β treatment of $CD4^+$ $CD25^-$ cells may lead to the induction of the transcription factor *FoxP3* and the generation of a regulatory phenotype. Furthermore, although classically described as being non-proliferative, recent evidence indicates that T_{Reg} can proliferate *in vivo* and are therefore capable of expanding in response to stimulating antigens.[13]

Whilst identification of T_{Reg} is associated with expression of CD25, this receptor is also expressed on normal activated T cells. However, in contrast to T cells that express CD25 during activation, T_{Reg} appear non-blastic and do not express other markers associated with recent activation. Recently, *Foxp3*, the gene encoding the transcription factor Scurfin, has been associated with the generation and function of murine T_{Reg} cells and does not appear to be expressed by normally activated T cells. Accordingly, Scurfin-deficient (*sf*) mice lack T_{Reg} cells and suffer autoimmunity and conversely, transgenic mice that over-express *Foxp3* have increased numbers of T_{Reg} cells and display increased immunosuppressive activity when compared to wild-type mice.[14,15]

During pregnancy, the mother's immune system tolerates the fetus, which expresses paternal major histocompatibility complex antigens. Current evidence suggests that the maternal immune system in humans is aware of the presence of this 'allograft', but fails to mount a classical

Received 13 January 2004; revised 5 March 2004; accepted 12 March 2004.

Correspondence and present address: Dr D.M. Sansom, Division of Immunity and Infection, University of Birmingham, Birmingham, B15 2TT, UK. E-mail: D.M.Sansom@bham.ac.uk

immune response;[16] the mechanisms behind this tolerance are still being sought. One important mechanism that has emerged in materno-fetal tolerance is tryptophan catabolism by dendritic cells (DCs). The breakdown of tryptophan by the enzyme indoleamine 2,3 dioxygenase (IDO) leads to suppression of T-cell responses. Strikingly, inhibition of IDO has been shown to lead to abortion of allogeneic, but not syngeneic, concepti in mice.[17,18] This strongly suggests that IDO activity is a key mechanism involved in materno-fetal tolerance in mice. Whilst studies in humans are limited, IDO has been detected in human decidual macrophages[19] and in placental villous explants, and has been shown capable of inhibiting human T-cell responses.[19] Intriguingly, IDO expression may also be influenced by cytotoxic T-lymphocyte antigen-4 (CTLA-4), a molecule expressed by T_{Reg} cells.[20,21] We therefore hypothesized that CTLA-4-expressing T_{Reg} may play a role in inducing maternal tolerance to alloantigens during pregnancy. To test this hypothesis, we have evaluated changes in T_{Reg} numbers during the course of normal human pregnancy. Our data show that T_{Reg} cells are significantly increased during pregnancy and return to lower levels post partum.

MATERIALS AND METHODS

Ethics approval for this study was provided by the Local Research Ethics Committee and all women gave their informed, written consent. Venous blood from healthy women (aged 19–42 years) was subjected to an automated full blood count, to measure numbers of lymphocytes, and to flow cytometry to assess proportions of lymphocyte subsets. The whole blood fluorescence-activated cell sorter (FACS) lysis method was used with phycoerythrin- (PE), fluorescein isothiocyanate- (FITC) and peridinin chlorophyll protein (PerCP) fluorochrome-conjugated mouse monoclonal antibodies to CD3, CD4, CD8, CD11a, CD25, CD45RO, CD45RA, CD69, HLA-DR (Becton Dickinson, Oxford UK) and CXCR3 (R & D Systems, Oxford, UK). Briefly, whole blood (50 μl) was stained with 5 μl labelled antibody for 30 min. Red cells were then lysed by addition of FACS lysing solution (Becton Dickinson) according to the manufacturer's instructions. Cells were washed once in phosphate-buffered saline and analysed. For comparison, whole blood was diluted in RPMI-1640 and activated T cells were generated by incubation for 3 days with the mitogen phytohaemagglutinin at a concentration of 25 μg/ml.

Suppression of alloresponses by T_{Reg}

Functional lymphocyte studies were carried out using mononuclear cells isolated from blood by Ficoll density gradient centrifugation. $CD4^+$ T cells were purified by immunomagnetic negative selection (Stem Cell Technologies, Meylan, France). $CD25^+$ $CD4^+$ cells were positively selected using anti-CD25 microbeads (Miltenyi Biotech, Bisley, UK). $CD4^+$ $CD25^-$ cells were collected from the washing steps, and the $CD4^+$ $CD25^+$ cells were subsequently retrieved from the beads.

DCs were cultured from unrelated donors using monocytes purified from blood by negative immunomagnetic selection (Miltenyi Biotech) and cultured for 6 days in RPMI-1640 medium containing 10% fetal calf serum with granulocyte–macrophage colony-stimulating factor (800 U/ml), interleukin-4 (500 U/ml) and standard antibiotics.[22]

Alloantigen stimulation experiments were performed using the cultured DCs. $CD25^+$-depleted $CD4^+$ T cells (1×10^5) were stimulated with DCs at ratios of 1 : 10 and 1 : 100 DC : T cells in 200-μl final volumes. T-cell proliferation was measured by [^{3}H]thymidine incorporation. The inhibitory effect of the $CD25^+$ $CD4^+$ T cells was assessed at the ratios shown.

Real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Oligo-dT-primed first-strand cDNA was synthesized with a SuperScript™ First-strand Synthesis System (Invitrogen, Paisley, UK) as a template for real-time PCR. PCR primer pairs and internal *Taq*Man probes for the genes of interest were as follows:

Foxp3 forward 5′-GAGAAGCTGAGTGCCATGCA-3′ and reverse 5′-AGGAGCCCTTGTCGGATGAT-3′. FAM probe 5′-AAAATGGCACTGACCAAGGCTTCATCT GT-3′; and β-actin, forward 5′-CCTGGCACCCAGCA CAAT-3′ and reverse 5′-GCCGATCCACACGGAGTA CT-3′ VIC probe 5′-ATCAAGATCATTGCTCCTCCTG AGCGC-3′.

The FoxP3 primers were used at final concentrations of 300 nM and 400 nM, respectively, and the probe at a concentration of 200 nM. For β-actin, primers were used at final concentrations of 20 nM and 70 nM, respectively, and the probe at a final concentration of 100 nM. Other components of the PCR mix were from the *Taq*Man Universal Master Mix (PE Applied Biosystems, Warrington, UK). Forty-five cycles of 95° for 15 seconds and 60° for 1 min were carried out using an ABI Prism 7700 Sequence Detection system. The relative quantity of mRNA was calculated by plotting a curve of threshold cycle (C_T) for FoxP3 and β-actin for each sample.

Statistics

Data were non-parametrically distributed. The two-tailed Mann–Whitney test was used to compare pairs of groups, with significance defined as $P < 0{\cdot}05$. The Kruskal–Wallis test was used to assess changes in the proportion of T_{Reg} cells with gestation.

RESULTS

Normal pregnancy is associated with an increase in $CD4^+$ $CD25^+$ T_{Reg} cells

Initially the percentage of T_{Reg} cells was evaluated (as a proportion of the total peripheral lymphocyte population) in pregnant individuals ($n = 63$) compared to non-pregnant controls ($n = 25$). This revealed (Fig. 1a) that the median percentage of $CD4^+$ $CD25^+$ T cells was doubled during pregnancy (8·9%, range 3·4–28·1%) compared to controls (4·4%, range 2·3–8·0%; $P < 0{\cdot}0001$). Analysis by stage of

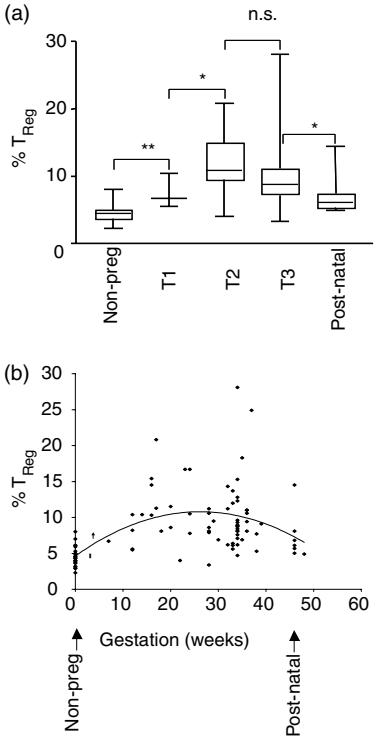

Figure 1. Changes in the blood T_{Reg} cell population size during pregnancy. Box and whisker plot showing $CD25^+$ $CD4^+$ T_{Reg} population as a percentage of total $CD4^+$ cells, giving medians, quartiles and ranges for each trimester (T1–T3). T1 = < 13 weeks, T2 = 14–26 weeks, T3 = > 27 weeks gestation, Postnatal = 6–8 weeks. Scatter plot with regression line showing percentage T_{Reg} for individual pregnancies at given weeks of gestation. $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; n.s., not significant (Mann–Whitney test).

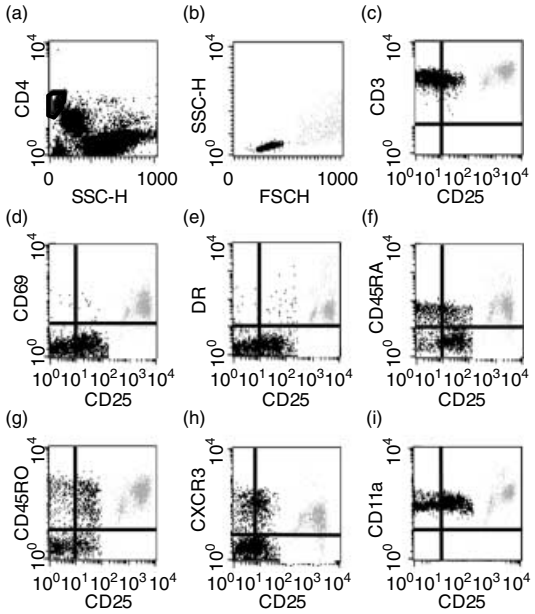

Figure 2. Three-colour flow cytometry analysis of $CD4^+$ $CD25^+$ cells during normal pregnancy. (a) Dot plot for fluorescence intensity of CD4 versus side light scatter showing gating for identification of $CD4^+$ lymphocytes. (b) Dot plot for forward versus side light scatter of gated $CD4^+$ lymphocytes demonstrating small size of these cells during pregnancy (black) and large size following mitogen stimulation (grey). (c) Dot plot for CD3 PE versus CD25 FITC of gated $CD4^+$ lymphocytes demonstrating that all of these cells are T cells ($CD3^+$) and that 22% during pregnancy (black) have weak CD25 expression (upper right quadrant) whilst all (grey) have strong CD25 expression after activation by mitogen. The quadrants are set according to isotype-matched, negative control antibodies. (d–g, i) Dot plots for CD25 PE versus CD69 FITC, DR FITC, CD45RA FITC, CD45RO FITC, CD11a FITC, respectively, for gated $CD4^+$ lymphocytes. (h) Dot plot for CD25 FITC versus CXCR3 PE for gated $CD4^+$ lymphocytes.

pregnancy showed a significant increase from the non-pregnant controls to the first trimester [4·4% ($n = 25$) versus 6·7% ($n = 5$); $P < 0{\cdot}01$] and from first trimester to second trimester [6·7% ($n = 5$) versus 10·9% ($n = 13$); $P = 0{\cdot}03$]. However, there was no significant change from the second to third trimester [10·9% ($n = 13$) versus 8·9% ($n = 45$); $P = 0{\cdot}06$]. The proportion of $CD4^+$ $CD25^+$ T cells was significantly lower 6–8 weeks following delivery than during the third trimester [8·9% ($n = 45$) versus 7·5%; $P = 0{\cdot}03$ ($n = 7$)] but remained higher than pre-pregnancy (7·5% versus 4·4%; $P < 0{\cdot}01$). A scatter plot of the distribution of results against gestational age is also shown (Fig. 1b). Analysis of variance using the Kruskal–Wallis test suggested that the variation in T_{Reg} cells with gestation was highly significant ($P < 0{\cdot}0001$). Since the total lymphocyte counts were not significantly different between the pregnant and non-pregnant women, the increased proportion of $CD4^+$ $CD25^+$ T cells was also reflected in increased absolute numbers.

To characterize further the phenotype of the $CD4^+$ $CD25^+$ T cells, FACS analysis was carried out. Representative FACS plots are shown in Fig. 2. Results for T_{Reg} from a pregnant woman are shown in black and for comparison results from control blood stimulated for 3 days with phytohaemagglutinin are shown in grey. The levels of CD25 observed in T_{Reg} were considerably lower than those seen in T-cell blasts. In addition to small size (Fig. 2b), evidence that these $CD4^+$ $CD25^+$ T cells were T_{Reg} cells rather than recently activated T cells included a lack of expression of markers associated with activation (CD69 and HLA-DR). This contrasted with high levels of expression of CD69 and HLA-DR amongst the activated lymphocytes (Fig. 2d,e). CD45RO was present on 34–52%, indicating previous exposure to antigen, with a reciprocal proportion positive for CD45RA, demonstrating that $CD25^+$ T_{Reg} were contained within both the CD45RO 'memory' population as well as the apparently naïve CD45RA cells. CXCR3, an important chemokine receptor for migration into allografts but also noted on regulatory T cells,[23] was found on 38–50% of T_{Reg} cells in pregnancy and at higher levels than in blasts. Overall this analysis showed marked differences between $CD4^+$ $CD25^+$ cells

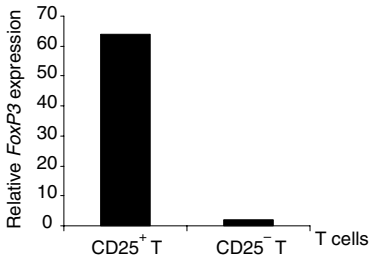

Figure 3. *FoxP3* is up-regulated in $CD4^+$ $CD25^+$ T cells. Quantitative PCR analysis was carried out for *FoxP3* and β-actin. Relative expression was calculated from the cycle threshold for *FoxP3* relative to an internal β-actin standard, for purified $CD25^+$ and $CD25^-$ T cells.

purified from peripheral blood during pregnancy and $CD4^+$ $CD25^+$ cells generated as a consequence of immunogenic stimulation. This indicated that the $CD25^+$ cells observed in pregnancy were not the consequence of classical stimulation *in vivo*.

T_{Reg} cells express *FoxP3* and suppress alloresponses

FoxP3 is now an established marker for T_{Reg} cells in mice and humans.[15] To provide further support for our hypothesis that $CD4^+$ $CD25^+$ T cells isolated during pregnancy represented T_{Reg} cells, the expression of *FoxP3* within these cells was investigated using quantitative PCR (Fig. 3). This revealed that *FoxP3* was highly enriched in $CD4^+$ $CD25^+$ T cells compared to $CD25^-$ T cells. These data were consistent with previous studies suggesting that *FoxP3* is selectively expressed in T_{Reg} and supported our contention that the $CD4^+$ $CD25^+$ cells observed in pregnancy were indeed T_{Reg} cells.

To establish that the $CD25^+$ T cells identified possessed regulatory function, their ability to suppress allo-responses in $CD25^-$ T cells was also analysed. Purified $CD25^-$ T cells were stimulated using allogeneic DCs and the effect of adding back $CD25^+$ T cells was assessed. This demonstrated (Fig. 4) that the $CD4^+$ $CD25^+$ cells isolated from healthy pregnant women were capable of suppressing [^{3}H]thymidine uptake in allogeneically stimulated lymphocytes by approximately 50%. Interestingly, no statistically significant difference in suppressive activity was found between five pregnant women and their non-pregnant controls. Overall these data suggest that the $CD4^+$ $CD25^+$ T cells isolated from pregnant women contain a regulatory population that on a per cell basis are of similar potency to those from non-pregnant controls, but which are present at a significantly elevated frequency.

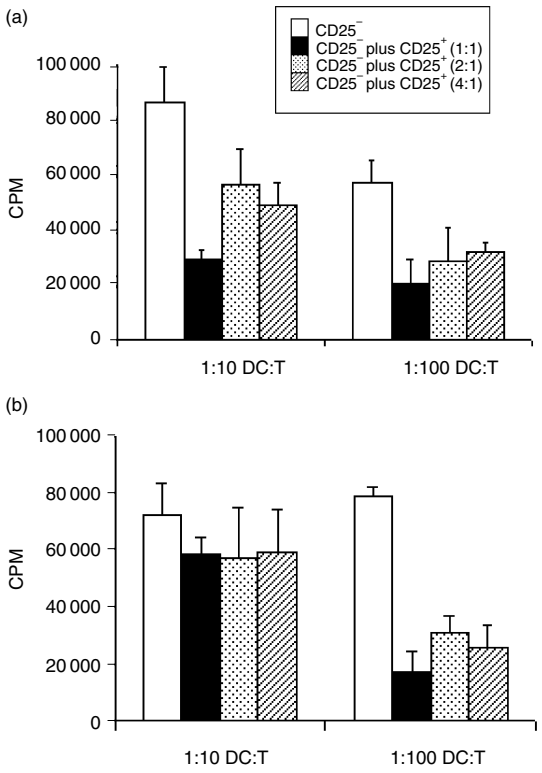

Figure 4. T_{Reg} from pregnant women suppress alloresponses. T_{Reg} cells from a pregnant (a) and a non-pregnant (b) woman were tested for their ability to inhibit proliferation of autologous $CD25^-$ T cells stimulated by allogeneic DCs for 5 days. The ratio of DCs to CD4 $CD25^-$T cells is shown on the *x* axis and the ratio of T_{Reg} to $CD25^-$ T cells is given for each bar. Responses (counts per minure; c.p.m.) represent the mean [^{3}H]thymidine incorporation plus the standard error of triplicate cultures. Data shown are representative of five separate paired experiments.

DISCUSSION

We have investigated the prevalence of the $CD4^+$ $CD25^+$ T-cell subset in human pregnancy, to determine whether changes in this subset might be relevant to materno-fetal tolerance. We hypothesized that T_{Reg} cells may play a role in modifying the maternal immune response to the feto-placental 'allograft' within the uterus. The dramatic and rapid increase in circulating T_{Reg} cells during early pregnancy, peaking during the second trimester at a time when trophoblast invasion of the maternal decidua is maximal, is consistent with this.[24] Equally, the decline in T_{Reg} cells postpartum is also consistent with the withdrawal of the immunological stimulus of the allograft. We have demonstrated that this population of $CD25^+$ cells can inhibit the induction of lymphocyte proliferation by a third-party allogeneic stimulus and is therefore likely to be capable of this function *in vivo*. Since *in vitro* studies suggest that cell–cell contact is necessary for T_{Reg}-mediated immunosuppression, we are also extending studies of blood lymphocytes to an assessment of T_{Reg} cells within decidua.

Our functional studies demonstrate that these $CD4^+$ $CD25^+$ lymphocytes suppress [^{3}H]thymidine incorporation in $CD4^+$ $CD25^-$ lymphocytes stimulated with allogeneic DCs by around 50%. These results confirm that the $CD4^+$ $CD25^+$ lymphocytes studied have T-cell

inhibitory activity, although, in our experiments so far, they do not appear any more potent than T_{Reg} cells from non-pregnant controls. However, given the increase in absolute numbers of T_{Reg} in pregnancy, one would predict that this should lead to a more immunosuppressed immune environment compatible with materno-fetal tolerance. An intriguing possibility is that the increase in regulatory cells reported here provides an explanation for the observation that a number of autoimmune conditions, such as systemic lupus erythematosus and rheumatoid arthritis, tend to remit during pregnancy.[25,26] Since, once activated, T_{Reg} do not appear to act in an antigen-specific manner, allospecific T_{Reg} may be able to regulate T cells of other specificities, including those promoting autoimmunity.

The mechanism by which T_{Reg} could modulate responses during pregnancy is not yet established. However, recent studies in mice have demonstrated that tolerance to alloantigens is essential for successful pregnancy. Studies by Munn *et al*. demonstrated that allogeneic, but not syngeneic, concepti were aborted in a T-cell-dependent manner when the enzyme IDO was inhibited.[18,27] Very recently studies have shown that soluble CTLA-4 or T_{Reg} expressing CTLA-4 can stimulate the up-regulation of IDO and the catabolism of tryptophan.[21] Our studies could now provide a link between IDO activity and CTLA-4^+ T_{Reg} cells in pregnancy. Thus increasing numbers of T_{Reg} could stimulate IDO expression in antigen-presenting cells and thereby decrease tryptophan levels. In support of this concept it has been shown that levels of tryptophan decline with each trimester during normal pregnancy.[28] These data fit well with the increase in T_{Reg} numbers observed in the present study.

In conclusion, the results presented here support our hypothesis, and it seems plausible that during pregnancy an increase in the population of T_{Reg} cells plays a role in maternal immune tolerance of her allogeneic conceptus. Studies are now in progress to characterize the phenotype and function of T_{Reg} cells in both normal and compromised human pregnancies in more detail.

Whilst this manuscript was under revision, the paper by Aluvihare *et al*. reported findings highly consistent with our own, using a murine model.[29] These data also show an increase in T_{Reg} in pregnant mice with gestation. However, they demonstrate that T_{Reg} are elevated irrespective of whether the fetus is allogeneic or syngeneic. Nonetheless, depletion of $CD25^+$ T cells resulted in the selective abortion of allogeneic concepti. Overall these data are highly consistent with our findings in humans and suggest that elevation of regulatory T cells in normal pregnancy provides immune protection for the fetus.

ACKNOWLEDGMENTS

The authors would like to thank the University of Birmingham Scientific Projects Committee for supporting this work. Y.Z. was funded by the BBSRC and the Wellcome Trust. D.M.S. was funded by the Arthritis Research Campaign and is an ARC Senior Research Fellow.

REFERENCES

1 Sakaguchi S. Regulatory T cells: key controllers of immunologic self-tolerance. Cell 2000; **101:**455–8.
2 Shevach EM. CD4+ CD25+ suppressor T cells: more questions than answers. Nat Rev Immunol 2002; **2:**389–400.
3 Read S, Powrie F. CD4 (+) regulatory T cells. Curr Opin Immunol 2001; **13:**644–9.
4 Graca L, Cobbold SP, Waldmann H. Identification of regulatory T cells in tolerated allografts. J Exp Med 2002; **195:**1641–6.
5 Wood KJ, Sakaguchi S. Regulatory T cells in transplantation tolerance. Nat Rev Immunol 2003; **3:**199–210.
6 Thorstenson KM, Khoruts A. Generation of anergic and potentially immunoregulatory CD25+CD4 T cells *in vivo* after induction of peripheral tolerance with intravenous or oral antigen. J Immunol 2001; **167:**188–95.
7 Hara M, Kingsley CI, Niimi M. *et al*. IL-10 is required for regulatory T cells to mediate tolerance to alloantigens *in vivo*. J Immunol 2001; **166:**3789–96.
8 Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. J Exp Med 1999; **190:**995–1004.
9 Yamagiwa S, Gray JD, Hashimoto S, Horwitz DA. A role for TGF-beta in the generation and expansion of CD4(+) CD25(+) regulatory T cells from human peripheral blood. J Immunol 2001; **166:**7282–9.
10 Liu H, Hu B, Xu D, Liew FY. CD4+CD25+ regulatory T cells cure murine colitis. the role of IL-10, TGF-beta, and CTLA4. J Immunol 2003; **171:**5012–17.
11 Nakamura K, Kitani A, Fuss I, Pedersen A, Harada N, Nawata H, Strober W. TGF-beta1 plays an amportant role in the mechanism of CD4(+) CD25(+) regulatory T cell activity in both humans and mice. J Immunol 2004; **172:**834–42.
12 Chen W, Jin W, Hardegen N. *et al*. Conversion of peripheral CD4+CD25– naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. J Exp Med 2003; **198:**1875–86.
13 Walker LS, Chodos A, Eggena M, Dooms H, Abbas AK. Antigen-dependent proliferation of CD4+ CD25+ regulatory T cells *in vivo*. J Exp Med 2003; **198:**249–58.
14 Khattri R, Cox T, Yasayko SA, Ramsdell F. An essential role for Scurfin in CD4+CD25+ T regulatory cells. Nat Immunol 2003; **4:**337–42.
15 Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 2003; **4:**330–6.
16 Sacks G, Sargent I, Redman C. An innate view of human pregnancy. Immunol Today 1999; **20:**114–18.
17 Mellor AL, Sivakumar J, Chandler P, Smith K, Molina H, Mao D, Munn DH. Prevention of T cell-driven complement activation and inflammation by tryptophan catabolism during pregnancy. Nat Immunol 2001; **2:**64–8.
18 Munn DH, Zhou M, Attwood JT. *et al*. Prevention of allogeneic fetal rejection by tryptophan catabolism. Science 1998; **281:**1191–3.
19 Kudo Y, Boyd CA, Sargent IL, Redman CW. Tryptophan degradation by human placental indoleamine 2,3-dioxygenase regulates lymphocyte proliferation. J Physiol 2001; **535:** 207–15.
20 Grohmann U, Orabona C, Fallarino F. *et al*. CTLA-4-Ig regulates tryptophan catabolism in vivo. Nat Immunol 2002; **3:**1097–101.

21 Fallarino F, Grohmann U, Hwang KW. *et al*. Modulation of tryptophan catabolism by regulatory T cells. Nat Immunol 2003; **4:**1206–12.
22 Hewison M, Freeman L, Hughes SV. *et al*. Differential regulation of vitamin D receptor and its ligand in human monocyte-derived dendritic cells. J Immunol 2003; **170:**5382–90.
23 Sebastiani S, Allavena P, Albanesi C. *et al*. Chemokine receptor expression and function in CD4+ T lymphocytes with regulatory activity. J Immunol 2001; **166:**996–1002.
24 Pijnenborg R, Bland JM, Robertson WB, Brosens I. Uteroplacental arterial changes related to interstitial trophoblast migration in early human pregnancy. Placenta 1983; **4:**397–413.
25 Cortes-Hernandez J, Ordi-Ros J, Paredes F, Casellas M, Castillo F, Vilardell-Tarres M. Clinical predictors of fetal and maternal outcome in systemic lupus erythematosus. A prospective study of 103 pregnancies. Rheumatology (Oxford) 2002; **41:**643–50.
26 Thurnau GR. Rheumatoid arthritis. Clin Obstet Gynecol 1983; **26:**558–78.
27 Friberg M, Jennings R, Alsarraj M. *et al*. Indoleamine 2,3-dioxygenase contributes to tumor cell evasion of T cell- mediated rejection. Int J Cancer 2002; **101:**151–5.
28 Schrocksnadel K, Widner B, Bergant A, Neurauter G, Schennach H, Schrocksnadel H, Fuchs D. Longitudinal study of tryptophan degradation during and after pregnancy. Life Sci 2003; **72:**785–93.
29 Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. Nat Immunol 2004; **5:**266–71.